PROC. ENTOMOL. SOC. WASH.
88(2), 1986, pp. 280–286

# DISCOVERY OF THE IMMATURE STAGES OF *PARASIMULIUM CROSSKEYI* PETERSON (DIPTERA: SIMULIIDAE), WITH A DISCUSSION OF A UNIQUE BLACK FLY HABITAT

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*Abstract.*—Larvae, pupae, and teneral adults of *Parasimulium crosskeyi* Peterson were collected in June 1984 from a hyporheic habitat adjacent to Wahkeena Creek, Oregon. An account of the collection and brief description of larvae and pupae are given. The immature stages of *Parasimulium* appear to be obligate members of the hyporheic zone. Morphology reveals a number of similarities with typical cavernicolous organisms, including apparent blindness of larvae and reduced cuticular pigmentation in both larvae and pupae. In addition to suspension feeding, larvae may be consuming organic detritus deposited in the substrate.

Dipterists have been interested in the enigmatic black fly genus *Parasimulium* Malloch, since the initial collection of adults over 70 years ago. Until recently, only eight male specimens were available for study, all but one collected by A. L. Melander in the Pacific Northwest (Washington, Oregon, and northern California). Included were the type species, *Parasimulium furcatum* Malloch, and a single representative of *Parasimulium melanderi* Stone. Peterson (1977), in a review of the genus, assigned the latter specimen to a new subgenus, *Astoneomyia,* and described two new species, *Parasimulium* (*P.*) *stonei* and *Parasimulium* (*P.*) *crosskeyi,* from the remaining material. In 1981, D. M. Wood and A. Borkent revisited the type locality of *P. crosskeyi* (Benson Park, Multnomah Co., Oregon), and collected large numbers of this presumedly rare black fly at adjacent Wahkeena Creek (Wood and Borkent, 1982). Their findings included discovery of the first females, and detailed information on behaviour of adults. Subsequently, additional information has been gathered for *P. crosskeyi,* and *P. stonei* (Peterson and Courtney, 1985; Borkent and Wood, 1986).

The papers by Peterson (1977) and Wood and Borkent (1982) have fostered considerable interest in locating larvae and pupae of *Parasimulium.* Because immature black flies possess a number of phylogenetically significant characters, discovery of these stages is a prerequisite to clarifying the position of *Parasimulium* within the Simuliidae.

Larvae, pupae, and teneral adults of *P. crosskeyi* Peterson were found in the hyporheic zone adjacent to Wahkeena Creek, Oregon (45°34′N, 122°07′W). The following is an account of the collection, a brief description of larvae and pupae, and discussion of ecological and phylogenetic considerations.

## Observations and Collections

Wahkeena Creek was visited on several occasions during 1984. *Parasimulium crosskeyi* adults were seen from May 26 to July 7, but abundances on these dates suggest that the period of activity is actually longer. Adults were most abundant during the first two weeks in June, and this may represent peak emergence time. Hundreds of *Parasimulium* adults were observed on June 11–12, primarily along the creek between the scenic highway and the southern border of Benson Park (Wood and Borkent's "section 4"). Behaviour of adults was as described by Wood and Borkent (1982), with males hovering or resting beneath leaves of bigleaf maple, *Acer macrophyllum* Pursh, red alder, *Alnus rubra* Bong., and other streamside vegetation. Adult black fly numbers were greatest at trees overhanging Wahkeena Creek, but some individuals were observed beneath *Acer* as far as 50 m from the stream. Flight times on June 11–12 generally agreed with Wood and Borkent's findings, with adults active from 0630 h to 2100 h. Male *Parasimulium* were found resting beneath the leaves of streamside vegetation long after sunset (ca. 2200 h).

At approximately 1200 h on June 12, large numbers of adult *P. crosskeyi* were observed near a seep below the Wahkeena Creek picnic area. The seep drains a seemingly large hyporheic zone (interstitial aquatic habitats below and lateral to the streambed) beneath the park, with Wahkeena Creek the ultimate source of water for the habitat. Water from the creek apparently percolates through coarse substrates lateral to the streambed, and continues via subterranean passageways under the picnic area. Water leaving the hyporheic zone drains into a shallow pool and re-enters Wahkeena Creek ca. 10 m from the seep. A variety of herbaceous plants, including stinging nettle, *Urtica dioica* L., bracken fern, *Pteridium aquilinum* (L.) Kuhn., and horsetail, *Equisetum telmateia* Ehrh., surrounded the seep outlet. Because *P. crosskeyi* adults were swarming beneath these low-lying herbaceous plants, instead of the typical woody trees and shrubs, the site appeared unique, and warranted further investigation.

The outlet of the hyporheic zone was excavated in hopes of finding the immature stages of *P. crosskeyi.* The habitat contained coarse substrates, primarily cobble-sized (sensu Cummins and Lauff, 1969) stones, which provided an extensive interstitial network, both below and above the water level. While rocks were removed from the seep, a net was held downstream to collect dislodged material. The current carried large amounts of woody and root-derived detritus out of the seep, and the net required periodic cleaning. Several trays of debris were checked for immature black flies, but only *Prosimulium esselbaughi* Sommerman were seen. However, each tray contained 1–2 teneral adult *P. crosskeyi,* which seemed to emerge from within the detritus. Because of the abundance of adults near the seep outlet, and the possibility of external contamination of samples, extreme care was exercised in collecting the last, and deepest (ca. 1 m into bank) sample. The sample contained no visible immature black flies, but did include 5 teneral adults. A close sex ratio of all "hyporheic" adults (2 males : 1 female), relative to samples taken elsewhere (ca. 10% females), was additional evidence that the site was unique. Debris from the last sample was retained for laboratory examination, in hopes of finding pupal exuviae of collected adults. This proved successful, as numerous exuviae, 2 male pupae, and 3 larvae of *P. crosskeyi* were found. Association of pupae was by examination of the pharate adult through the pupal integument; genitalia were well developed and assignable to *P. crosskeyi.* Larvae possessed poorly developed gill histoblasts, so association with pupae was not possible; however, structure of the hypostoma was unusual, and resembled that of early instar *Parasimulium* larvae reared from eggs (Borkent and Wood, 1986).

## Description of Immature Stages

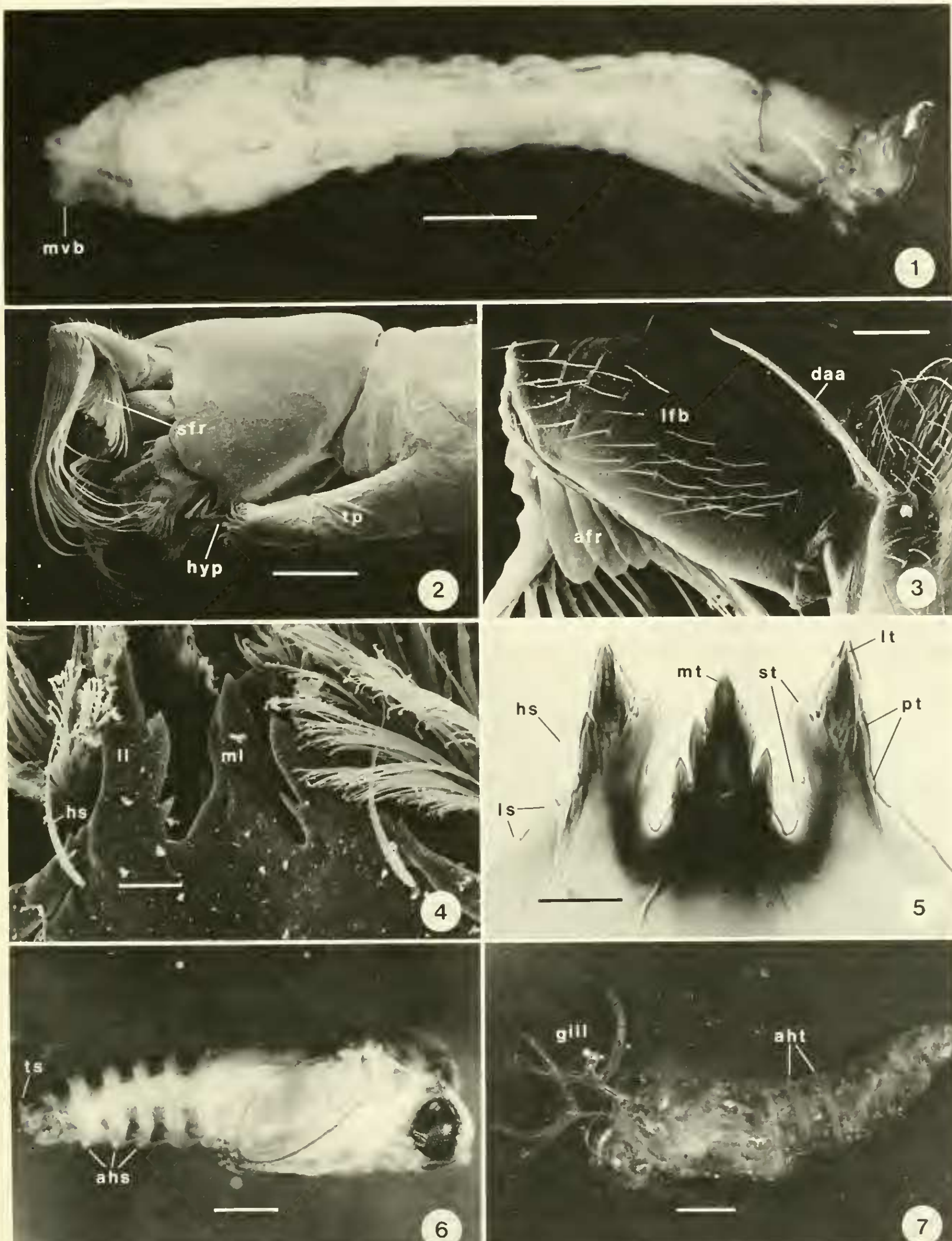
A full description of *P. crosskeyi* larvae and pupae awaits collection of additional material, particularly last instar larvae. I therefore present only a general description of the more salient features of collected specimens, with emphasis on characters that may be of phylogenetic importance.

Numerous larvae have been examined (including additional specimens collected at Wahkeena Creek on January 5, April 19, May 16, June 10, and June 24, 1985). Total length from 1–5 mm, with the latter probably representing the penultimate or antepenultimate instar. Cuticle is unpigmented, except for a few heavily sclerotized structures on the head (Fig. 1); stemmata not apparent. Labral fans well developed (Fig. 2); secondary fan rays as in *Prosimulium,* with tips arranged in a straight line. Antenna relatively short, extending to ca. ¾ length of labral fan base; proximal and medial articles (sensu Currie, in press) short, with distal article comprising 80% of antennal length (Fig. 3). Hypostoma (Figs. 4, 5) with 3 distinct lobes; lateral lobe with pronounced apical (lateral) tooth, 2 sublateral teeth (with innermost tooth small, and outermost tooth large), 2 small paralateral teeth, and 5 or more lateral serrations; median lobe slightly less pronounced than lateral lobes, and separated from lateral lobes by deep slots; median lobe with apical tooth and 3 lateral tines, which, beginning on the posterior right margin, alternate to the apex; margins of apical median, lateral, and outermost sublateral teeth serrated. Postocciput nearly complete, enclosing cervical sclerites. Postgenal cleft moderately shallow, but visible only after staining. Thoracic proleg elongate, its apex extending to a point anterior to the hypostomal groove. Abdomen with pronounced mid-ventral bulge (Fig. 1). Gut contents of one specimen included coarse sand grains, leaf particles, and a larval chironomid head capsule.

Two male pupae and several pupal exuviae were examined. Total length from 2–3.5 mm. Pupal integument transparent (Figs. 6, 7). Gill filaments translucent, inflated and with shallow annulations; gill consists of an elongate base and two strongly divergent primary trunks; dorsal trunk simple and ventral trunk branching horizontally into two filaments of subequal length. Abdominal tergites 3 and 4, each with 8 anteriorly-directed hooks along posterior margin. Tergite 9 with a pair of curved, moderately long, dorsally-directed terminal spines. Posterior margins of abdominal sternites 5–7, each with 2 anteriorly-directed hooks, situated on postero-ventrally directed tubercles. Cocoon a thinly-woven sack covering the abdomen and thorax, with a poorly defined anterior margin.

## Discussion

Hyporheic insect faunas are well documented (see Hynes, 1983; Williams, 1984) and include a variety of Diptera, Ephemeroptera, and Plecoptera. Some taxa are considered obligate members of the subterranean community (Stanford and Gaufin, 1974), but most are transient, remaining in the hyporheos only until reaching a larger, presumably less vulnerable, size. The habitat is therefore dominated by early instars of typical surface benthos. Black flies are rarely included in discussions




Figs. 1–7. *Parasimulium crosskeyi*. 1, Photomicrograph of whole larva, showing mid-ventral bulge (mvb). Scale = .5 mm. 2, Scanning electron micrograph of larval head and anterior thorax, showing secondary fan rays (*sfr*), hypostoma (*hyp*) and thoracic proleg (*tp*). Scale = .2 mm. 3, Scanning electron micrograph, showing labral fan base (*lfb*) and larval antenna (distal article = *daa*). Scale = 50 μm. 4, Scanning electron micrograph of larval hypostoma, showing hypostomal sensillum (*hs*), lateral lobe (*ll*) and median lobe (*ml*). Scale = 20 μm. 5, Photomicrograph of larval hypostoma, showing lateral serrations (*ls*), hypostomal sensillum (*hs*), median tooth (*mt*), sublateral teeth (*st*), lateral tooth (*lt*) and paralateral teeth (*pt*). Scale = 20 μm. 6, Photomicrograph of male pupa (gills absent), showing terminal spines (*ts*) and recurved abdominal hooks on sternites 5–7 (*ahs*). Scale = .5 mm. 7, Photomicrograph of pupal exuvia showing gill and location of recurved abdominal hooks on tergites 3–4 (*aht*). Scale = .5 mm.

of hyporheic environments, mostly because of rather conservative views about larval feeding habits. Simuliids are usually considered strict suspension feeders and, to a lesser degree, scrapers of attached periphyton. In fact, larvae are generally capable of engaging in several feeding strategies, depending on food resources (Currie and Craig, in press).

A number of simuliids are facultatively hyporheic. Peterson (1970) reported that *Prosimulium onychodactylum* Dyar and Shannon [complex] often pupates within gravels of the stream bottom. He also predicted this behaviour for *P. susanae* Peterson, which has been corroborated under laboratory conditions (D. A. Craig, unpubl. data). These examples pertain to stationary, non-feeding stages, and entrance into the hyporheic zone may accrue only defensive advantages. Black fly larvae entering this environment may have to cope with dramatically different food resources than in surface habitats. Larvae of *Gymnopais* Stone, scrapers inhabiting small, arctic-alpine streams, are capable of surviving in the hyporheos (Currie and Craig, in press). The organism retreats into the substrate during periods of excessive or low flow, and apparently becomes a detritivore. Early instar *Gymnopais* larvae are uncommon in surface samples, but may frequent the hyporheic zone, even during normal flow conditions (D. C. Currie, pers. comm.). Collections at Wahkeena Creek indicate that *Simulium pugetense* (Dyar and Shannon) [complex] (collected in January, April, and May 1985) and *Prosimulium esselbaughi* may enter the hyporheic zone. The presence of these suspension-feeding larvae in the Wahkeena Creek hyporheos may reflect the site's unique characteristics. The poorly-sorted, coarse substrate and extensive interstitial network probably allow surface benthos easy access into the subterranean habitat. Future research may uncover additional simuliid taxa, the larvae of which are capable of surviving in similar hyporheic environments.

Unlike the above simuliids, *Parasimulium* appears to be an obligate member of the hyporheic zone. Morphology of the immature stages of *P. crosskeyi* demonstrates a number of similarities with typical cavernicolous organisms (Howarth, 1983). Apparent blindness of larvae, and unpigmented cuticle in both larvae and pupae, are among these subterranean adaptations. Ecological characters seem equally affected by this life style. Period of adult emergence is somewhat protracted in *P. crosskeyi,* extending at least 5 weeks. *Parasimulium stonei* exhibits a similar pattern (Peterson and Courtney, 1985). This asynchrony of emergence could be a response to dampened environmental cues, relative to surface habitats. Wahkeena Creek is a short (<1.5 km), spring-fed system, which exhibits much less seasonality than most Pacific Northwest streams. The extended period of emergence in *P. crosskeyi* may reflect subtle changes in water temperature or chemistry; however, establishing this with certainty requires detailed examination of ecological parameters. An additional consideration pertains to food availability. The Wahkeena Creek hyporheic habitat contains an abundance of woody and root-derived organic debris. Fine particulate organic matter (FPOM) derived from this debris is a potential food for the fully-fanned *Parasimulium* larvae, provided subterranean current velocities are high enough to suspend particles in the water column. No data on underground currents is available, but relative discharge at the collection site, and adjacent seepages, and the presence of two filter-feeding simuliid taxa (*S. pugetense, P. esselbaughi*), suggest that flow is sufficient to maintain a fauna of suspension feeders. Although *Parasimulium* seems capable of this feeding strategy, analysis of the gut contents of one specimen indicates that larvae may be "deposit" feeding on the microbial and invertebrate (e.g. chironomid) fauna associated with detritus. Future gut analysis of larvae, and closely monitored behavioural studies should provide valuable insights on the feeding strategies of *Parasimulium.*

The discovery of immature stages of *Parasimulium* will surely rekindle the controversy surrounding higher classification of the Simuliidae. Character analysis incorporating data on *Parasimulium* larvae and pupae will provide a test of the classification used by several authors (Crosskey, 1969; Peterson, 1977; Wood and Borkent, 1982; and others), which places *Parasimulium* in a subfamily by itself, the Parasimuliinae. Such analysis may, instead, indicate that the genus simply constitutes an aberrant prosimuliine branch. It is evident that the immature stages of *Parasimulium* and those of the Prosimuliini share a number of character states; however, many of these represent the presumed plesiomorphic condition and, as such, are of little value in reconstructing phylogenies (Wiley, 1981). In addition, ecological (e.g. habitat) specialization may distort interpretations of character polarity. Elucidating the relationship between *Parasimulium* and the rest of the Simuliidae requires a thorough phylogenetic analysis. Discovery of the immature stages of *P. crosskeyi* will provide an impetus for such examination, and foster a renewed interest in hyporheic research.

## Acknowledgments

I am indebted to D. M. Wood, Biosystematics Research Institute, for sharing his knowledge of *Parasimulium* and directing me to the candidate larval habitat, the hyporheic zone. For numerous enlightening discussions about simuliid biology and critical review of the manuscript, I thank D. A. Craig and D. C. Currie, University of Alberta. I am grateful to L. A. Keeth for eager field assistance on the June collecting trip, and N. H. Anderson, Oregon State University, for kindly providing laboratory facilities during much of the 1984 field season. Finally, I thank District Ranger Gene Zimmerman, Mt. Hood National Forest, for granting permission to invade Wahkeena Creek picnic area with emergence traps, drift nets, and other entomological paraphernalia. This project was funded by Natural Sciences and Engineering Research Council of Canada grant No. A5753 to D. A. Craig.

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